Lide Laboratories Inc.

Medical Diagnostic Products

Diagnostic Stains

HANSEL Stain Directions for Use:

HANSEL® Stain, one-minute technic, is formulated for use as an aid in differentiating eosinophils and neutrophils using a quick, inexpensive, clinical microscopic procedure. HANSEL Stain is adaptable for various secretions such as nasal, urine, sinus, aural, conjunctival, salivary, bronchial and gastrointestinal.Determining the cytologic picture through the examination of the nasal secretions can differentiate an allergic condition (eosinophil infiltration) from a bacterial infection (neutrophil infiltration). Determining elevated levels of eosinophils in urine (eosinophiluria) can aid in the diagnosis of many associated conditions. When cytologic specimens are stained with the HANSEL Stain formulation and magnified, eosinophils and eosinophil granules appear red and neutrophils and mucous secretions appear blue. The result is a contrasting, colorimetric procedure for cytologic evaluation. A noninvasive secretion examination can be an easy, first diagnostic step, especially in children. Leukocytes are the major cellular components of inflammatory and immune responses and include neutrophils, eosinophils, T and B lymphocytes, monocytes, and basophils. All these cells have a specific function from antibody production to the destruction of bacteria. Neutrophils are classically thought to be critical to host defense against bacteria. Although eosinophils have similar morphology and cellular actions as neutrophils, they do not appear to have an important role during bacterial infections. However, eosinophils are produced and play a central role in host defense for conditions such as allergic reactions, bronchial asthma, helminthic infections, and other disease states. In many cases, cytological examination of secretions can be a useful aid in establishing a diagnosis of an allergy, infection or other condition. In allergy, the pathologic picture has traditionally been characterized by eosinophilic and basophilic infiltration and edema. For example, nasal secretions of patients with allergic rhinitis are often rich in eosinophils. The degree of secretion eosinophilia is proportional to the severity of the symptoms and reactions. In milder cases of nasal allergy, eosinophils are present in comparatively small number, while in hay fever, large masses of cells may be demonstrated. By contrast, most acute, subacute and chronic infections have been characterized by neutrophilic infiltration, consequently neutrophils are found to predominate in secretions. For instance, in the resolution stage of the common cold, neutrophils are present in large numbers and eosinophils are only scattered. Infections frequently complicate the allergic picture and acute or subacute infections should be recognized and treated as such. Subacute bronchial infections may occur with asthma, and subacute maxillary sinusitis in allergy may require antibiotic treatment. If an infection complicates allergy of the respiratory tract, the eosinophils completely disappear from the secretions during the infectious stage but return after the infection is resolved. If resolution is delayed or does not occur the neutrophils persist. Determining the presence of elevated eosinophil levels in urine can aid in the diagnosis of other conditions. Eosinophiluria has been found with drug induced acute interstitial nephritis (AIN), eosinophilic cystitis, atheroembolic renal disease and schistosomiasis. Urinary eosinophilia has been associated with glomerulonephritis, acute prostatitis and acute cystitis as well as rejection of renal allografts. HANSEL Stain has proven to be highly superior for the identification of urinary sediment eosinophils, and the predictive value of eosinophiluria. While other stains are inhibited with a pH of less than 7.0, HANSEL Stain has been shown to be highly sensitive. In fact, Hansel Stain is the only stain cited for detecting eosinophils in urine according to the Laboratory Test Handbook , 3rd Edition 1994, and the Medical Laboratory Observer, May 2002.

PREPARATON AND STAINING OF SAMPLES

Several methods may be employed in the collection of secretion from the nose for smear examination. Secretions are most easily and readily collected by having the patient blow the nose on waxed paper or cellophane handkerchief. This gives a specimen which represents only nasal secretion or a collection of both nasal and sinus secretion. Small crusts from the septum and vestibule of the nose should be avoided. If no secretion is available from blowing the nose, it may be necessary to remove it by swabbing with a cotton applicator, suction cannula or aspiration. Specimens may also be taken separately from each nostril. A secretion may also be collected in a specimen bottle at the time of an acute exacerbation. Bronchial secretion is readily obtained from coughed specimens; ocular secretion from the inner canthus.

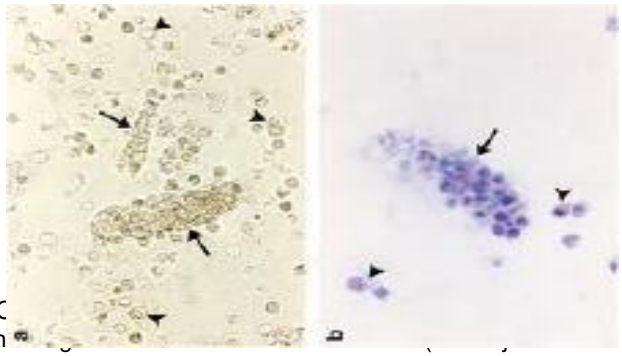
All specimens should be smeared on a clean microscope slide. Staining will be better if the secretion is teased out evenly on the slide. Prepare 2 or 3 slides if there is enough secretion.

Dry smears in air or dehydrate with methyl alcohol. When samples cannot be stained immediately, they may be fixed with methyl alcohol. This will suffice as a preservative for several days. If it is desirable to delay staining for a prolonged period, slides may be fixed with a Carbowax-Ether-Alcohol Solution.

Flood slides completely with HANSEL Stain and allow to stand 25 - 30 seconds, giving the longer period of time to thicker or milky smears. DO NOT DIP. Add distilled water to take up stain, and allow to stand 30 seconds. Pour off stain and rinse stain with distilled water to remove excess stain. Quickly rinse slide with 95% ethyl or methyl alcohol. Drain off and dry in air. Wipe back and edges of slide.

Urine Specimens: Prepare slides for urine cytology using approximately 10 mL of freshly voided, clean catch, midstream urine. Centrifuge at 2000 rpm for 5 minutes. Decant supernate. Pipette a specimen from sediment onto a clean, glass slide air-dry or dehydrate with 95% methyl alcohol. Cytospin procedure works well. Flood completely with HANSEL Stain and allow to stand 30-45 seconds. Add distilled water to take up stain, and allow to stand 30 seconds. Pour off stain and rinse stain with distilled water to remove excess stain. Quickly rinse slide with 95% ethyl or methyl alcohol. FOR URINE SAMPLES, complete a final rinse with distilled water. Drain off and dry in air. Wipe back and edges of slide.

a) Unstained urine sediment showing leukocyturia with cellular casts.



(b) Hansel Stain of urine sediment showing both free eosinophils and eosinophils in casts.
FROM: Acute allergic interstitial nephritis and eosinophiluria M Kaye (retired) and R F Gagnon

ADDITIONAL STAINING INFO

In examination of specimens, th a 12.5x to 15x eyepiece). Use a moderately strong, clear, colorless light. Do not use a blue filter in lamp or sub-stage disc. Keep sub-stage light shutter open. Binocular scopes with a magnification of 100x may be satisfactory. For higher power magnification (100x), oil immersion and cover slips should be used. If alcohol is used in excess, it will wash the blue stain out of the neutrophils and cause them to look pink. Pink neutrophils present a clear homogeneous cytoplasm. If the stain is too dark to identify the cells, excess may be removed by further clearing with methyl alcohol.

There are no true granules in neutrophils and they should be readily distinguishable from eosinophils which stain red with deep red granules and a predominant blue nucleus. Free red granules often appear from broken eosinophils.

It is often not possible to draw conclusions from the examination of a single smear. Therefore it may be necessary to make an examination of several smears, especially if the specimen does not contain sufficient material or acute or chronic infection complicates the picture.

In addition, variations in secretion type and morphology of cells may require modification of the staining technic. For instance, new cells seen early in an infection have good morphology compared to late stages where the cells are breaking down. For study of bacteria in cells (phagocytosis) and for details of epithelial cells, a lighter stain is desirable. Also, if the cells are predominately eosinophilic and basophilic (mast cells), additional use of methyl alcohol during the final rinse should produce very bright red and clearly visible granules. HANSEL Stain is highly adaptable to various situations.

INTERPRETATION OF RESULTS

Results of nasal secretion examinations are often evaluated using a semi-quantitative grading score on a 1 to 4 scale for both eosinophils and neutrophils. Other times results are simply quantified as the percent of visible eosinophils (related to the proportion of neutrophils), where levels > 80% are considered suggestive of asthma or allergy.

In urine analysis, eosinophils are generally reported as the percentage of eosinophils per 100 white cells present in the preparation. Following the original protocol, 1% (or more) eosinophils is regarded as positive. Another source defines eosinophiluria as >5 percent of urine leukocytes and eosinophilia as the presence of more than 500 eosinophils per microliter of blood. However, there is an uncertain relationship between eosinophiluria and peripheral blood eosinophilia.

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